CHITINASE PRODUCTION BY CHITINOLYTIC BACTERIA FROM CATFISH

(C*larias gariepinus*)

BY

ONIBOKUN, ADEOLA ELIZABETH

13PCQ00506

DEPARTMENT OF BIOLOGICAL SCIENCES

SCHOOL OF NATURAL AND APPLIED SCIENCES

COVENANT UNIVERSITY

MAY, 2015

CHITINASE PRODUCTION BY CHITINOLYTIC BACTERIA FROM CATFISH

(C*larias gariepinus*)

BY

ONIBOKUN, ADEOLA ELIZABETH

13PCQ00506

A DISSERTATION SUBMITTED TO THE MICROBIOLOGY PROGRAMME OF THE DEPARTMENT OF BIOLOGICAL SCIENCES SCHOOL OF POSTGRADUATE STUDIES

COVENANT UNIVERSITY, OTA,

OGUN STATE, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

AWARD OF MASTER'S DEGREE IN MICROBIOLOGY

MAY, 2015.

DEDICATION

I dedicate this project work to God Almighty, The Giver of life, my Sustainer, Provider of all resources and most of all the supplier of my strength, and wisdom without whom this work would not become a reality.

DECLARATION

I, ONIBOKUN ELIZABETH ADEOLA, hereby declare that this project work was carried out by me in the department of biological sciences, school of natural and applied sciences, college of science and technology under the supervision of Dr A. A. Ajayi.

This project work has not been submitted anywhere else for a degree award. The ideas are majorly products of a research conducted by me. All ideas from other authors have been duly acknowledged.

ONIBOKUN ELIZABETH ADEOLA ----------

Researcher Signature and Date

CERTIFICATION

We certify that this project work was carried out by ONIBOKUN ELIZABETH ADEOLA, 13PCQ00506 in the Microbiology programme of the Department of Biological Sciences, School of Natural and Applied Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria.

---------- ----------

Signature Date Signature Date

Dr. A. A Ajayi Dr. E. E. J. Iweala

(Project supervisor) (HOD, Biological Sciences)

---------- ----------

Signature Date Signature Date

Prof. Charles Ogbologo Prof. Bola Adeniyi

(Dean, School of Postgraduate studies) (External Examiner)

ACKNOWLEDGEMENT

I return all the glory to God for the success of this work. My gratitude also goes to my supervisor, Dr A. A Ajayi for her constructive criticisms, motherly advice and dedication throughout the period of this work. I will also use this medium to appreciate all my lecturers, Prof. Egwuari, Prof. Nandita , Dr Oranusi, Dr Owolabi, Dr lawal, Dr Olaseinde, Dr Ayepola, Dr Omonhinnin for all their support and encouragement. I also appreciate the efforts of all technologists in the Department of Biological sciences. May God reward you all.

Finally, I appreciate the efforts of my parents for their financial contributions, my siblings, friends, colleagues and Miss Adedeji, Odia Trust for their numerous contributions to the success of this work.

It is my prayer that the favour of God will not depart from your lives. Thank you and God bless you all in Jesus name. Amen.

TABLE OF CONTENT

LIST OF PLATES

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Chitinases are hydrolytic enzymes that breaksdown the glycosidic bonds in chitin. Chitin is a component of the cell walls of fungi and exoskeletal elements of some animals (including worms and arthropods), therefore chitinases are generally found in organisms that either needs to reshape their own chitin or dissolve and digest the chitin of fungi or animals. The importance of chitinase in industries cannot be overemphasized as it has been applied in agriculture; as a biopesticide for control of plant fungi infections, in medicine; as indicators of fungi infection, and in waste management; for biodegradation of fish waste. The African catfish (*Clarias gariepinus*) which plays host to these chitinolytic bacteria is very readily available and easy to cultivate thus providing a cheap means for the production of chitinase in commercial quantity. Bacteria populations Isolated from catfish were screened on colloidal-chitin agar medium. Chitinase production was determined by zones of hydrolysis produced after 96h of incubation at 37^{o}C. The activity of Chitinase was determined by measuring the amount of reducing sugar released using Dinitroslicylic method. Partial purification of chitinase was carried out by Ammonium Sulphate precipitation. The optimum conditions for the chitinase activity were determined using a number of parameters such as temperature, pH, effect of substrate concentration and the time of heating over 30min. Optimum conditions were therefore ascertained at a temperature of 50^{0}C and a substrate concentration of 0.15g for chitinase produced by bacteria spp (isolate code 17 and 36) while pH 5.5 was obtained for isolate code 36 and pH 6.0 for isolate code 17. The Michaelis – Mentens constant (Km) which is also known as the dissociation constant is the substrate concentration at half maximum velocity. Calculated from the Lineweaver-Burk plot, the apparent Km values for the hydrolysis of chitin by chitinolytic bacterial isolate code 36 and isolate code 17 were approximately 0.09mg/ml and

0.007mg/ml respectively. Isolation of DNA and PCR amplification was carried out on the chitinolytic bacteria used for the study and one of the bacteria was identified as a member of the genus *Bacillus*. This study established that species of *Bacillus* inhabiting the gut and skin of the African catfish can be used to produce chitinase in appreciable quantity. The study has diversified the use of catfish for enzyme production rather than for consumption only. The catfish gut which constitutes a large portion of the fish waste will also serve as a cheap source for chitinase production.